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Evaluating the Effects of Genetic Drift and Natural Selection in *Drosophila melanogaster*

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This laboratory provides a "hands on" experimental approach to illustrate evolution in a semester-long study using red-eye and white-eye phenotypes of *Drosophila melanogaster*. Students set up and maintain small and large fly populations for several generations to observe the effects of genetic drift and natural selection. They record the phenotypes, calculate allele frequencies and at the end of the semester, submit a formal laboratory report on this experiment which includes chi-square tests, and graphs of allele frequencies, heterozygosity and Fst values. They gain valuable practical, analytical, and writing experience from this experiment.

Keywords: evolution, *Drosophila melanogaster,* natural selection, evolution, genetic drift, population size, heterozygosity

Introduction

This laboratory has been adapted from one developed at the University of Toronto (Goldman, C., 1991). It is currently used in the second year Evolution class at Dalhousie University, which is a required class for all Biology Majors. *Drosophila melanogaster* has proven to be an ideal organism for an undergraduate laboratory in evolution because it is relatively easy to maintain and has a short life cycle. It is often difficult to find "hands on" experimental laboratories to illustrate concepts for evolution. However, because of the short generation time of Drosophila, this experiment works in one semester, and is an effective means of observing evolution as it is occurring. Although the methodology and objectives are preset, this lab offers students a chance to have a bit of "real" laboratory research experience. Flies wake up and escape, cultures get contaminated, and yes, you do have to come in over the study break to look after your flies. At the end of the experiment, the students have to analyze their data and write a formal paper on their experiment. They gain experience in finding and using research articles on which to base their expectations and to evaluate their results. I have broken up the due dates for components of the report. Students submit the Methods and Materials the week that they set up their fly cultures, the Introduction a week or two after that, and the rest at the end of the semester. The students seem to appreciate a more manageable assignment at the end of the semester, and I find they have a greater depth of understanding of the study while they are doing it, instead of after they write the report at the end.

Timing

One thing to consider is that because it is an ongoing semester -long experiment, it does take up a bit of time, and other labs need to be organized around the fly lab schedule. Our labs are two hours long. We do an introductory lab during which we go over administrative details of the laboratories but also use the time for the students to get acquainted with the flies, using the dissecting microscope and aspirator, and making a practice culture. This only takes about 60 – 90 minutes in total. The second scheduled lab time is spent entirely on setting up the fly cultures. In alternating weeks the removal of the adult flies should take about five to ten minutes at most. I usually have students do this as they are coming in so that most groups have this done before the scheduled lab begins. Two weeks from the initial set up of the cultures, the entire lab is spent on the first transfer. Some groups are much quicker than others. The second and third transfers require between 30 and 60 minutes for the students to complete. Students get much faster as the semester goes along and as their confidence and experience increases. The final fly lab is spent doing the phenotype counts of the F_4 generation, and recording the allele frequencies and heterozygosities for the small groups in Excel and discussing how to do the final report. I then post the data on the class website for students to use in their results.

Student Outline

The purpose of this study is to provide you with an opportunity to perform and evaluate a long term experiment to illustrate evolution using living organisms. You will track the phenotypes (allele frequencies) of your populations and compare the results with other groups in your lab. You will look for evidence of evolution in the form of genetic drift or natural selection by the changes in allele frequencies and further data analysis. You should be able to see which evolutionary forces have a greater effect on populations that differ in size. Read through the appropriate sections in your textbook and lecture notes for more background information, and look up research articles in the library for the more detailed understanding required in writing your laboratory report.

Genetic Drift

Unlike other forces of evolution, genetic drift is non-directional. It is the result of the random sampling of alleles at each generation, and its effects are most prevalent in small populations, or populations that are below an effective population size. This has been demonstrated in the following examples. The Flour Beetle (*Tribolium confusum)* has become a standard subject for laboratory experiments and has a body color of either brown or black, which is governed by two alleles at a single gene locus. The results of an experiment by Rich et al (1979) demonstrate the effects of genetic drift in these insects. In the experiment, populations of 10, 20, 50 and 100 individuals were set up. Each population began with an equal ratio of black to brown alleles. A change in allele frequencies occurred in all population sizes, but with the most extreme fluctuations (fixation at a frequency of one or elimination of the allele) occurring in the smallest population size of 10. In the second example, Buri (1956) performed an experiment using small populations of *Drosophila melanogaster* having equal frequencies of the two alleles *bw* and bw^{75} at the brown locus. In many of the populations the alleles became randomly fixed for one or the other allele. Interestingly, if you took the average, the overall allele frequency of all the populations was close to the starting frequency of 0.5, but the individual population allele frequencies varied widely. The results for both of these experiments are consistent with the expectations for genetic drift.

Natural Selection

For natural selection to occur there must be variation among individuals that is inherited and allows some of these individuals to be more successful at survival, mating, or reproducing than others. Unlike genetic drift which is random, natural selection will tend to produce a directional change in allele frequencies from generation to generation. If one allele consistently experiences greater success, then that allele will become more prevalent over time in that population. It is important to remember that while drift can behave differently in each population, the results of natural selection are consistent from population to population.

Dawson (1970) observed an example of natural selection while studying a recessive lethal allele in *Tribolium* beetles. The recessive allele caused death in homozygotes and was therefore not passed on to offspring as often as the dominant viable allele. In repeated experiments this caused a steady decrease in the lethal allele and increase in the viable allele in succeeding generations.

Geer and Green (1962) observed evidence of natural selection in colonies of *Drosophila melanogaster*. Their study supported previous experiments that concluded that mate selection was visually dependent, but they also found that when red-eyed male flies were competing with white-eyed males for mates, the wild-type males had greater mating success.

Materials and Methods

Study Organism: Drosophila melanogaster

The common fruit fly *Drosophila melanogaster* is often used in laboratory studies. The fruit fly is easily cultured and its generation time (egg to adult) is only two weeks at 21-23^{o}C. It makes an ideal organism for laboratory studies because cultures occupy little space and the fly is big enough to easily observe the phenotypic traits under a dissecting microscope. We will be working with eye-color phenotypes for this lab.

Life Cycle of Drosophila

There are four distinct stages in the life of the fruit fly: egg, larva, pupa, and adult. A fresh culture of *D. melanogaster* can produce new adults in two weeks: 2-3 days for the embryo stage, 6 days in the three larval stages, and six days in the pupal stage. The adult fruit fly is fertile after 12 hours but reaches maximum fertility between days 3 and 7, although the adult fly may live for several weeks and remain fertile during that time.

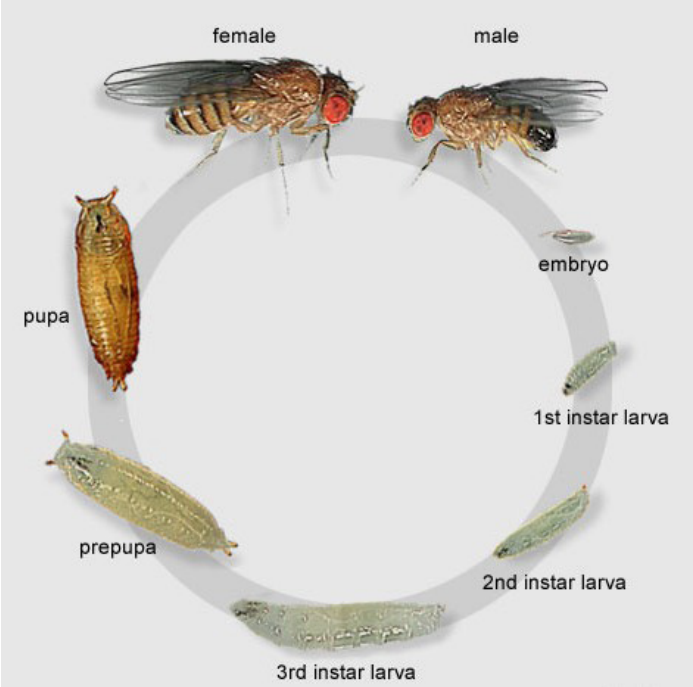

Figure 1. *Drosophila melanogaster* Life Cycle (K. Weigmann et al, 2009)

The larva hatches one day after the egg is laid. The larva molts twice shedding its cuticle, mouth hooks, and spiracles. The larva is called an instar during the periods of growth before and after molting and there are three such instars in Drosophila. Finally the cuticle hardens and darkens to become the puparium, inside which metamorphosis occurs. After metamorphosis, the adult fly emerges from the puparium. It is important to note that newly emerged flies are light in color with folded wings and a longer appearing abdomen. Within a few hours the coloring darkens, the wings expand, and the abdomen rounds up a bit more. This is important to remember when you are sexing your flies.

The Red-eye (Wild-type) and White-eye phenotypes of Drosophila

Your original stocks of flies (F_0) will all be **true breeding** or **homozygous** for either the red-eye or white-eye phenotype. Note that the red-eye color is the wild type and white-eye color is the mutant type. Red-eye is dominant to white-eye. The gene for eye color is carried on the X chromosome in Drosophila. Females are XX and have two copies of the gene for eye color. Red-eyed females therefore have the genotype w^+w^+ and white-eyed females are *ww*. There are no heterozygous females in the original stock populations.

Male Drosophila have only one X chromosome and one Y chromosome, so that red-eyed males have the genotype w^+ Y and white-eyed males are *wY*. When you look at a male fly, you can tell which eye color allele it has.

Remember that after the parental generation, a red-eyed female can have the genotype *w+w+* **or** *w+w,*

Sexing

The sex of each fly can be determined by examining the flies using the dissecting microscope. The easiest method is by looking at the body of the flies. The end of the abdomen is more rounded and darker in males, and lighter and more pointed in females. The males are also slightly smaller than the females. Remember what can be confusing about this method is that flies that have newly emerged from the pupal casing tend to be lighter, so it is helpful to switch to a higher magnification and focus on the front legs. There are sex combs on the front legs of the male but not on those of the female.

Culture of the Flies

The fly colonies are grown in large and small culture vials. The culture medium is made up of commercially prepared flakes which when combined with water, contains nutrients for normal larval development and also allows the yeast to grow. The

culture media is prepared simply by adding equal amounts of tap water and dried flakes. Fresh media must be prepared for each new generation and will be set up by the lab technician. For the large bottles, approximately 30 mL of water is added to 30 mL of media flakes. For the small populations, 15 mL of water is added to 15 mL of flakes in a small vial.

Handling Your Flies

Be gentle! Use a soft bristled paintbrush for moving them around.

- For your first lab session and the initial set up of your large and small cultures, your Instructor will have pre-cooled (unconscious) true-breeding flies of each eye color for you to use. They can go directly onto your sorting plate when your equipment is set up and you are ready to begin.

- During the semester, for your transfers, you must chill the flies yourself. Rap your culture vial on a soft surface (lab manual, stack of cardboard, etc.) to tap the flies down to the bottom of the vial before inserting the aspirator tip carefully past the foam plug. By *gently* sucking on the rubber hose attached to the aspirator you can "vacuum up" the flies from your culture bottle into a "transfer" vial.

- Put the transfer vial into the ice bucket. Chill the flies for *at least five minutes* to make sure that they are unconscious and likely to stay that way when you observe them under the dissecting scope. Tap down the vial intermittently to ensure that the flies are at the bottom area of the vial that is immersed in the ice; they tend to hide at the top near the black rubber stopper.

- Tap out a few unconscious flies onto the glass sorting plate (glass square placed over one Petri dish filled to the top with crushed ice). This will help to keep the flies cool and unconscious when you are examining them under the microscope. Remember to wipe the surface of the glass plate often to keep the surface dry.

- Do not get your flies wet!! Condensation often builds up on the glass sorting plate and in the vials. If flies are put into these wet vials they will die. Before putting flies onto the sorting plate or in a vial or large bottle, check to see that there is no condensation.

Setting Up Your Cultures

Equipment needed for each small group of three people:

Styrofoam ice bucket and ice	Dissecting microscope
Labels (use masking tape)	Glass sorting plate (1)
Petri dish (1)	Culture bottle for large population (1)
Paint brush (1)	Culture vial for small population (1)
2 aspirators with rubber hoses and vials	Fly morgue

Set up one small population first which will consist of eight flies:

2 wild-type females	2 white-eye females
2 wild-type males	2 white-eye males

Remember that the stock bottles contain flies that are true breeding, so the females are homozygous and we use equal numbers of flies with each trait to start off with an initial allele frequency in F_0 of $\boldsymbol{p = 0.5}$.

1. Wash your hands with soap and water. Disinfect your work surface area.
2. Set up your equipment and let your lab TA or the Instructor know when you are ready for flies. You will be given about 10-20 wild-type or white-eyed flies from the stock bottles.
3. Sex the flies under the dissecting microscope. On the sorting plate, under the microscope, sort out males and females. Take time to ensure that you are familiar with the differences between the sexes, so that you are confident in your sexing. Check with your TA if necessary.
4. Aspirate any 2 wild-type males and any 2 wild-type females into a clean, transfer vial.

   Do not put the flies into their culture vials yet. Otherwise by the time you add the white-eye flies the wild-type will have revived enough to escape during transfer.

5. Label the vial so you know this is the culture transfer vial. Aspirate any extra or escaping flies into the spare transfer vial and label it "extra".
6. Repeat steps 2 to 5 for white-eye flies.
7. Now chill all eight flies together in the transfer vial in the ice bucket for *at least* five minutes.
8. Once the flies have been "out cold" for the five minutes, use a weigh paper to gently transfer your flies from the aspirator transfer vial into the culture vial. **Important!** Keep the culture vial on its side until all the flies wake-up, otherwise the flies will fall to the bottom and stick to the culture media and die there.
9. Label your culture vial with your group's name (you can be inventive!) or the initials of each member of your group. Place your culture vial in the tray labelled with your lab day and time. Lab staff will place it in the fly room until next week.

Set up one large population, which will consist of 40 flies:

10 wild-type females	10 white-eye females
10 wild-type males	10 white-eye males

10. Repeat the procedure outlined above.

    Sexing and counting will have to be efficient, or else flies will wake up on the sorting plate. We recommend that one student sort the flies while another aspirates chosen flies up, and a third student keeps track of the number of the two sexes (use the data sheet provided) and vacuums up the flies that are waking up or are extras. Sort no more than about 10 - 20 flies at a time, and keep the remaining flies yet to be sorted on ice. Once you have all your flies, chill them in the transfer vial on ice all at the same time for at least five minutes, and then carefully transfer them to the large culture bottle which you have labelled. Record your phenotype data and the allele frequencies on the sheets provided in your lab manual. *Remember* to keep the culture vial on its side until all the flies wake-up, otherwise the flies will fall to the bottom and stick to the culture media.

Clean-Up

ALL used equipment (basically everything except the microscopes) goes to the main sink area to be washed and autoclaved. At the end of each lab, discard unwanted flies into the jar (morgue) located by the sink. Fill used culture jars to the top with water and replace the foam stopper so no flies escape. Clean all working surfaces with disinfectant to avoid bacterial or mite contamination.

Your flies will mate and lay eggs within a couple of days.

In One week:

After one week you should vacuum out the parents, so the adults do not mate with newly emerging offspring and the only flies present in your cultures next week will be progeny.

In Two weeks:

You will **randomly sample** 8 flies from the small population and 40 from the large population to be the parents for the next generation as follows.

1. Pick up your culture bottles you set up two weeks ago. Since you removed the parental flies a week ago all the flies in your culture vials will be their progeny. If a population has failed inform your TA or Instructor. They will let you know how to handle this situation. You may have to begin a new culture, or there may be some extra cultures you may use.
2. Randomly select the new parents. For example, take the small culture jar started with 8 flies:

   Gently aspirate as many flies as possible into the transferring vial and place this on ice until the flies are immobilized for at least five minutes.

   Place the flies on the sorting plate. Take the first 4 males and 4 females.
3. Record the eye-color phenotypes of the 8 randomly selected flies on the data sheet.

4. Use the transfer technique to place the 8 flies in a fresh culture vial.
5. Label the vial with a name for your group or the group member initials.
6. Set up the large population. In this case you must randomly select 20 males and 20 females. Remember that it is easier to sort about 20 flies at a time.
7. Dispose of excess flies from the previous cross in the morgue, unless otherwise instructed by your TA. **Clean up** your used culture vials and bottles.
8. Count and record F_1 ***phenotypes***.

   Calculate the ***allele frequencies*** and ***expected heterozygosities (2pq)*** at each transfer.

   Record your data in the tables provided.

For the Next Six Weeks:

Every second week for the next six weeks you will be repeating the same procedures as outlined above. You will randomly sample your two populations (small and large), count the phenotypes and calculate allele frequencies, and record all your data on the data sheets. It is your and your group's responsibility to ensure that the random samples during this period are carried out correctly.

In the following data sheets: Please note:

(1) In the F_0 parental generation, the female flies are homozygous, therefore $p_f = 0.5$.

(2) $p_{all} = (2/3)p_f + (1/3)\, p_m$

(3) Calculate one **final** chi-square value in the table.

DATA SHEETS for the Small Population of *Drosophila melanogaster*

PHENOTYPE OBSERVATIONS		**Small Population** (Total 8 flies)			
Gender	**Eye Color** **F_0**	**F_1**	**F_2**	**F_3**	**F_4**
male	red				
	white				
female	red				
	white				

ALLELE FREQUENCIES **Small Population** (8 flies)		**Generation Number**				
		F_0	**F_1**	**F_2**	**F_3**	**F_4**
Males	# red-eyes	2				
	p_m	0.5				
Females	# white-eyes	2				
	q^2	---				
	$p_f = (1 - q)$	0.5				
Population	p_{all}	0.5				
Expected Heterozygosity (*2pq*) (Use p_{all} and q_{all})		0.5				

χ^2 test Small Population	Red-eye Males	White-eye Males	Red-eye Females	White-eye Females	
Observed					
Expected					
Obs-Exp					
$\frac{(\text{Obs-Exp})^2}{\text{Exp}}$					$\chi^2 =$

DATA SHEETS for the Large Population of *Drosophila melanogaster*

PHENOTYPE OBSERVATIONS		**Large Population** (Total 40 flies)			
Gender	**Eye Color** F_0	F_1	F_2	F_3	F_4
male	red				
	white				
female	red				
	white				

ALLELE FREQUENCIES **Large Population** (40 flies)		**Generation Number**				
		F_0	F_1	F_2	F_3	F_4
Males	# red-eyes	2				
	p_m	0.5				
Females	# white-eyes	2				
	q^2	---				
	$p_f = (1 - q)$	0.5				
Population	p_{all}	0.5				
Expected Heterozygosity ($2pq$) (Use p_{all} and q_{all})		0.5				

χ^2 **test Large Population**	Red-eye Males	White-eye Males	Red-eye Females	White-eye Females	
Observed					
Expected					
Obs-Exp					
$\frac{(Obs-Exp)^2}{Exp}$					$\chi^2 =$

Formal Fly Lab Report

General Information

Your fruit fly experiment will be written up in the form of a scientific paper. The Methods and Materials section, and Introduction section will be handed in on separate due dates from the rest of the report. *Please note:* each section (excluding the results) of your lab report must be separately submitted and receive an acceptable rating from the Turnitin program to receive a grade for that section. The *"Turnitin*" link is found on the class BLS site (Select "*Labs*" and "*Fly Report*").
Your Fly Lab Report will be graded according to the guidelines presented in the lab manual, and those in the required writing book by Karin Knisely *A Student Handbook for Writing in Biology*. 3rd Ed., 2009 Sinauer Associates.

General Formatting Details:

You *will* be evaluated on your grammar and presentation. No title page necessary, can be single or double spaced, can be single-sided or double-sided, use size 12 point font. Be clear and organized and concise in your writing. Proof read! *Italicize all scientific names (species and genus)*. Write numbers out as words except when greater than ten or when used in a measurement or calculation (chi-square values etc.).

Materials and Methods

This is the section you will write first and hand in ahead of the other sections. This is the **final** Methods and Materials section you will hand in. Write it as though you have completed the entire term long study. We are writing it now because (a) it will give you a really good grasp of what you are doing in the lab and (b) it will be one less thing to have to write at the end of the semester when you have a million other things to get done!

Include a descriptive **title** for your report with the name of the organism involved. The title should reflect the study you did. Provide enough information in this section so that another researcher could repeat the study. Summarize the methods including the materials used in paragraph form - do not give lists of instructions. Include relevant information on the study organism (i.e., why were fruit flies used?). Outline the procedure used throughout the study.

Yes, you do need to reference your lab manual (can cite once), and it is the only reference that you need for this section.

This section should be a maximum of 1.5 pages (single spaced) in length.

Introduction

In this class, this is the section you will write second, and will hand in ahead of the other sections. As stated in the Knisely (2009) text, you should start off with general information and then get more specific. You will need to include:

- **The background of the evolutionary forces** (*must* include citations)
  - Include explanations or definitions of important concepts - you can include secondary textbook sources for definitions.
  - State the question(s) or hypothesis which you are addressing
  - Explain why the study was done (in the big picture, justify the experiment).

- **Outline of the approach used** to address the main question (i.e.; the experiment)
  - Summary of experiment and the expectations of results- you *must* include citations from three primary research articles for the expectations

- Results do not go in this section. Follow the triangle format with base at top ~ general information leading to more specific, ending with the hypothesis for the experiment/study at the point of the triangle at the bottom.
- *At least* ***three primary research references*** are required for this section.
- The introduction should be a maximum of 1.5 pages (single spaced) in length.

Abstract

Write this section last, although it is usually placed at the beginning of your report. The abstract should summarize the entire paper in a single paragraph. The abstract should be a maximum of 0.5 pages (single spaced) in length.

What are the main questions? What experiment and analysis was done to address this? What were the major results? What were the major conclusions?

Results

You must include a *written results section* before you present your graphs and chi-square tables. You should refer to your tables and figures within the written section. Organize your figures and tables in a logical order, reflecting the order in which you discuss them.

Please note all graphs should be done using a computer program such as Excel. Each figure should be only a half a page in size. All tables and figures should be numbered, labelled appropriately and have a proper descriptive title or caption. For figures, number and descriptive title or caption go at the bottom. For tables, number and descriptive title or caption, go at the top.

Data Analysis for this lab consists of four steps:

1. Computing the **allele frequencies** in your populations.
2. Testing (chi-square test) for **significant change** in allele frequencies across generations.
3. Computing the **variation** present in the populations.
4. Computing **divergence among populations.**

1. Computing Allele Frequencies

For each generation you should record the numbers of red-eye and white-eye flies and calculate the frequency of the red-eye allele. This data goes on your data sheets provided in the lab manual.

Recall that eye color is sex linked in Drosophila. This means we have to calculate the allele frequencies separately for males and for females.

Males are XY. There is no allele on the Y chromosome, so for sex-linked traits, the frequency of red-eye alleles, p, in males is equal to the phenotypic frequency.

Females are XX. For females, we can only estimate the wild-type allele frequency since we cannot distinguish heterozygotes from homozygous wild types. From the Hardy-Weinberg equation we know the frequency of white-eye female flies is equal to q^2 and so we can compute q, the white-eye allele frequency and then we can estimate the frequency of wild-type alleles in females as $p = 1 - q$.

Combine the allele frequencies for males and females to estimate the population-wide allele frequency. Since females carry two alleles for eye color and males just one, the population frequency is computed as a weighted average of male and female frequencies.

So, for our example:

In the F_1 generation you count three red-eyed males out of four.
Frequency of the **wild-type allele in males** is p_m =3/4 = 0.75.

In the F1 generation you count two red-eyed females out of four.
Frequency of **white-eye females** is $\boldsymbol{q^2}$ = 2/4 = 0.5
Frequency of **white-eye allele** in **females** is q = = 0.71
Frequency of **wild-type allele** in **females** is p_f = 1 - 0.71 = 0.29
Frequency of wild-type allele in the whole **population** $\boldsymbol{p_{all}} = (2/3)p_f + (1/3)\, p_m$
$\boldsymbol{p_{all}}$ = [2(0.29) + 1(0.75)] / 3 = 0.44

Calculate and record your allele frequency data in the Table provided.

2. Chi-Square Tests: Testing For Significant Change in Allele Frequencies Across Generations

In the results section, include two *chi-square tests* to determine whether the allele frequency in your lab group's populations changed significantly over the course of the semester. **Remember to use numbers rather than frequencies in your tests.** Compare the final allele frequency of your populations to the starting allele frequency. Did the frequency of the red allele go up or down? Test whether this difference is significant as follows:

Expected numbers are based on your initial allele frequencies of $p_{all} = 0.5$, $q_{all} = 0.5$

If there was no change in allele frequency, then under random mating, we would expect $p^2 + 2pq$ of the females to have red eyes and p of the males to have red eyes.

Observed numbers are from your F_4 generations.

In the written section, you must state the chi-square value (χ^2), degrees of freedom (*df)*, probability value (P) and make a clear statement regarding whether your observed values were significantly different from the expected. Make a statement if you rejected or failed to reject your hypothesis.

3. Computing the Genetic Diversity or Heterozygosity Present in the Populations

The amount of genetic diversity in a population can be measured by the heterozygosity (H_e) or frequency of heterozygotes of that population. The frequency of heterozygotes in your population can be calculated from your data assuming Hardy-Weinberg equilibrium, and would be equal to **2pq**. Remember to use the **p*(all)*** and **q*(all)*** values you have calculated for each generation. The highest value for the frequency of heterozygotes is 0.5, when the allele frequencies are also at 0.5.

4. Fixation Index or Fst (F-statistics) Calculations

Fst was originally proposed by Sewall Wright, and is a way to measure the reduction in the heterozygosity of sub-populations relative to the total population. You will need to obtain two measures of heterozygosity to calculate the Fst value. All of the data you need can be obtained from the class data sheets from your lab day and time that are posted on the class web site. Each of the small groups in your lab time has calculated the expected heterozygosity for their small and large populations in each generation. The first measurement needed in the Fst calculation is the **heterozygosity (H_e) within populations.**

$H_{e\ (within\ populations)}$ = the average value of *2pq* of different sub-groups for each generation.

$$H_{e\ (within\ populations)} = \frac{\text{2pq values for all sub-groups at one generation}}{\text{number of fly sub-groups in your lab}}$$

The $H_{e\ (within\ populations)}$ value for each of the four generations has already been calculated for you and is found on the data sheets posted on the web site.

The next data you need is the **heterozygosity for the pooled populations** or $H_{e\ (pooled)}$ for each of the four generations. Use the values of **p*(all)*** and **q*(all)*** for all the sub-groups from your lab day and time. Pool the p*(all)* and q*(all)* values for all the small populations find the averages, calculate 2pq, and then do the same for all the large populations.

$$p \text{ ave} = \frac{p \text{ for all fly groups in the lab at one generation}}{\text{number of fly groups in your lab}}$$

$$q \text{ ave} = \frac{q \text{ for all fly groups in the lab at one generation}}{\text{number of fly groups in your lab}}$$

$$H_{e\ (pooled)} = [2(\boldsymbol{p} \text{ ave})(\boldsymbol{q} \text{ ave})]$$

Now use these values $H_{e\ (within\ populations)}$ and $H_{e\ (pooled)}$, to calculate F_{ST} for each generation and for each population size.

$$F_{ST} = \frac{H_{e\ (pooled)} - H_{e\ (within\ populations)}}{H_{e\ (pooled)}}$$

The values for F_{ST} can range from 0 if there is maximum heterozygosity in the subpopulations, to 1, when the allele frequencies in the subpopulations are fixated.

Include Four Graphs in the results section: Each graph should be only a half a page in size.

Changes in allele frequency:

1) *Plot* the allele frequency (Pall) in each generation for all the small cultures (one graph). You should also include an average line for the average allele frequency.
2) *Plot* the allele frequency (Pall) in each generation for all the large cultures (another graph). You should also include an average line for the average allele frequency. Highlight your own group's data in both figures.

Genetic Variation He

3) *Plot* the average amount of genetic variation (He within) present in the small populations at each generation (use class data). *Plot* the average amount of genetic variation (He within) present in the large populations at each generation (use class data, both lines on one graph).

Population divergence Fst

4) Calculate and *plot* Fst for the small populations at each generation (use class data). Calculate and *plot* Fst for the large populations at each generation (use class data, both lines on one graph).

Appendix

This is marked as part of the results section but goes at the end of the report. Include the following raw data tables: 1) group phenotype observations; 2) group allele frequencies; 3) class allele frequencies; 4) class He; 5) class Fst.

Note: The chi-square tables and allele frequency graphs all go into the Results section.

Discussion

Go back to your Introduction - what were the main questions you were trying to answer?

Expectations: In this section you ***must have citations from primary sources*** (research journal articles) to establish your expectations.

You need to back up your expectation statements with references. You need to interpret your results.

- How would you expect the allele frequencies to behave under genetic drift and natural selection? What did you see in your results? What were your conclusions?
- What did you expect to see in the small and the large populations? What were your results and conclusions?
- What did you expect to see in the chi-square tests? What were your results and conclusions?
- Support your conclusions with your results. Do not use the word "prove".
- Discuss experimental error or inconsistencies in the results (do not use the term "human error").
- Put your work in the context of the broader evolutionary context--population genetics in the real world. What questions would you ask next?

Yes, you need at least three primary references for this section (and they can be the same ones used in your Introduction). You can use **subheadings** to structure your discussion logically.

The discussion should be a maximum of two pages long (single spaced).

How to Reference

Please use the Name-Year System described by Knisely (2009).

You need to reference **at least three primary** (journal) articles. You can use your textbook (secondary source) as a reference but it is *not* considered to be a primary article.

You also need to reference the lab manual (especially for the Materials and Methods section).

Remember to include page numbers used in all your references!

Examples - please note that these are fictitious examples but are written in the proper style required for this lab report. I had fun making them up - do you recognize any of the names?

Lab Manual: Lidenbrock, P. 2008-2009. Genetic Drift in Dinosaurs. BIOL 2040, Biology Department, Jules Verne University.

Journal: Crichton, M. 1982. Recovery of *Procompsognathus* DNA from Mosquitoes in Amber. *Journal of Fossilized DNA*.10:(1): 117-125.

Book: Malcolm, I., Grant, A, Sattler, E. 1989. *Chaos Theory in Jurassic Park.* Halifax: InGen Press. 35 p.

Materials

Equipment needed for lab of 24 students working in small groups of three people:

Drosophila melanogaster Red-eyed flies and white-eyed flies. We use two large cultures of each phenotype for each of our lab groups of 21-24 students. We keep our cultures going throughout the year, so do not necessarily order flies on a regular basis. More is always better!

(8) Styrofoam ice buckets. I recycled containers from shipments to research labs, they have an inside measurement of 22 x 12 x 16 cm (*l* x *w* x *h*)

(8) Dissecting microscopes and possibly Transformers – depends on your dissecting microscope

(8) Glass sorting plates (measuring approximately 10 cm x 10 cm just covering the Petri dish with a slight overlap)

(8) Petri dishes (90 x 15 mm) – use the deeper half to hold more ice.

(8) Large culture bottles for large populations with prepared media and yeast and foam plugs

(8) Small culture vials for small populations with prepared media and yeast and foam plugs

(8) Fine-Tip Brushes

(8) Containers of disinfectant solution

(8) Holders for the small vials (Otherwise they tend to fall over easily and get broken.) Ours were made from small wooden blocks with two circular holes drilled in them to support the small vials (9 x 4 x 4 cm (l x w x h)).

(16) Aspirators with rubber hoses and transfer vials. You should have a larger supply than just the 14 – we try to have at least three times this amount because this equipment must be washed and autoclaved between uses. Sometimes they get broken or plugged up with culture media.

(1) Fly morgue: This is a jar filled with water and a thin layer of olive or corn oil to cover the surface. We just have one and clean it out every week or more often, depending on the number of flies being euthanized.

(16) Sheets of weigh paper to transfer unconscious flies into the culture vials.

Access to ice (we use crushed ice)

Incubation room:
We have a small room off the laboratory that has been fitted with separate controls for heating and cooling. It is maintained at a fairly constant temperature of 21 - 23 degrees Celsius.

Notes for the Instructor

Media

Our technician makes up the vials with media for the cultures the day of or a day before the students need them. It is important to use fresh yeast (check the expiration date), and only one grain is needed for the small vials and two or three for the large culture jars. Use equal amounts of flakes and water. Too much water leads to a sloppy mixture, prone to fungus or bacterial contamination. We always try to use sterile techniques as much as possible to avoid contamination. For the large bottles, approximately 30 mL of tap water is added to 30 mL of media flakes. For the small populations, 15 mL of water is added to 15 mL of flakes in a small vial.

Equipment

We started leaving the rubber tubes attached to the aspirators since with repeated attachment and removal by students; the metal pipe tends to get pushed down and displaces the filter. The filter is the only barrier that prevents you from inhaling some flies, so it is important that students check the filter before each use.

Students should never share aspirators for hygienic reasons.

Repair of filters – I have found that the cotton cloth (in our first aid kit) works well. I just pull the metal tube out of the stopper, place one or two layers of a square of cloth over the tube, and push it back into place.

Try to store the brushes bristle side up in a jar or lay flat, otherwise the bristles get bent out of shape and are almost unusable.

If you have multiple lab sections, remember to have extra supplies to allow for washing and autoclaving time. We do not usually use soap in the culture jars.

Fly Crosses

I usually set up several extra fly cultures for each lab day during the initial set up of cultures. Most of the failures seem to occur during this first week for some reason. If a group's culture fails, they could just begin with the initial setup again in two weeks and be one generation short, but I find for morale purposes, having the extra cultures works out much better! Then they are right on schedule.

Safety and Stock Cultures

We stopped using ether completely even for technician maintained stock cultures. The transfer of flies into new stock cultures is done every three to four weeks by rapping the culture jar to knock flies to the bottom, speedily removing the foam plug and inverting a new culture jar on top, and allowing the flies to fly up into the open mouth of the new jar. This process can be sped up by next flipping both jars while keeping the open ends together and rapping them on the bench surface, effectively knocking the flies into the new culture jar, and quickly replacing the foam plugs.

Warning – it does take a bit of practice – be prepared to have a few escapees!

Acknowledgements

The laboratory is adapted from a lab developed at the University of Toronto (Goldman, C., 1991). Thank you for allowing us to present it here and use in our undergraduate laboratories. The Drosophila life cycle image is used with the kind permission of C. Klämbt.

Also thanks to Dr. Robert Latta for help with the data analysis requirements of this report and Chris Corkett for the initial organization of the laboratory setup. We are continually grateful to our TAs and for the calm and efficient help of our technical staff, past and present, Bob Jordan and Doug Mitchell, without whom we could never get 147 students and over two thousand flies and associated lab equipment working efficiently together every week!

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Weigmann, K., R. Klapper, T. Strasser, C. Rickert, G. Technau, H. Jäckle, W. Janning and C. Klämbt: FlyMove-a new way to look at development of Drosophila. **http://flymove.uni-muenster.de/Homepage.html?http&&&flymove.uni-muenster.de/hometxt.html** . Accessed April 2011.

About the Author

Elizabeth Welsh has a BSc. in Biology (McMaster), an MSc. in Molecular Biology and Genetics (Guelph), and a BEd. in Science Methods (Dalhousie). She has seven years experience in Research and Clinical laboratories in the Departments of Neuroscience at McMaster University in Hamilton, and Pathology at Women's College Hospital in Toronto. She was a professor in Biology at the Teacher's College in Truro for two years and the Senior Instructor in the first year Biology Majors Laboratories at Dalhousie University for twelve years. She has also taught Genetics and Biology for non-majors at Mount Saint Vincent University. Elizabeth is currently a Senior Instructor at Dalhousie University for the second year core class in Evolution, and is developing a non-majors Biology class.

Appendix

Equipment and Supplies needed and Suggested Suppliers and Costs

Item	**Suggested Supplier and Cost** (in Canadian dollars)
Drosophila melanogaster. White-eyed flies for 100 students	Ward's 87 V 6633 $22.45
Drosophila melanogaster. Red-eyed flies for 100 students.	Ward's 87 V 6621 $22.45
Instant Drosophila Medium, Blue, 1 L (blue dye makes it easier to see the larvae)	Ward's 38 V 0594 $8.69
Suction Tube Aspirator (includes transfer vial & rubber tube)	Ward's 10 V 0175 $14.06
Fine-Tip Brushes	Ward's 15 W 3846 $9.81 pkg of 12 (I have also purchased paint brushes from the dollar store as well – just try to get soft bristles).
Large culture bottles (We inherited ours, glass, 250 mL from Sun Life Technologies $3 US each)	The fly research lab in our department uses washed and sterilized glass juice bottles.
Small culture vials (Glass shell vials height 95 mm, 30 mL capacity)	Canada Wide Scientific (Kimble) Mfg.# 60931-8 Cat.# 951-500-18 $164.74 pkg of 144
Foam plugs	Ward's 18 W 4960 $ 4.06 pkg of 10
Petri dishes 90 x 15 mm	Ours are from Fisher, although they no longer carry the 90 mm size. The 100 mm size will work fine just enlarge the glass plate a bit. Fisher Scientific S33580 $7.15 or Wards 18 W 7101 $7.19 pkg of 20

Glass sorting plates (approximately 100 x 100 mm, 3 mm thick) covering the Petri dish with a slight overhang.	I had ours made at a local company "Admiral Glass" in Halifax. The cost to cut and polish the edges of 28 glass plates was $90.
T36® Disinfectant solution purchased in 4 L containers and divided up into 500 mL spray bottles.	Canada Wide Scientific Cat. # 308-700-24 $83.25 (can probably use other types – this one is carried in our Biology store).
Weigh paper (500 sheets per box)	Fisher-Scientific 3" x 3", cat No. 09-898-12A
Yeast	Small packets at any grocery store $1- $2
Fly morgue	Any approx 500 mL size jar with lid – fill with water and small amount of cooking oil to cover the surface

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